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PRENATAL MORTALITY IN PEROMYSCUS WITH SPECIAL REFERENCE TO ITS BEARING ON REDUCED FERTILITY IN SOME INTERSPECIFIC AND INTERSUBSPECIFIC CROSSES*

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INTRODUCTION

THE study of sterility in hybridization has occupied the attention of biologists for many years. Numerous investigations have demonstrated a wide range of mechanisms responsible for the reduced fertility. These mechanisms include: (1) sexual isolation, copulation not occurring because of the lack of mutual attraction of different forms; (2) mechanical isolation, copulation being difficult on account of the physical incompatibilities of the reproductive organs; (3) gametic isolation, spermatozoa failing to reach the eggs or to penetrate into the eggs; and (4) inviability of the hybrids, fertilization having taken place, with death of the zygote at some stage of development (Dobzhansky, 1941). It is apparent that the relative importance of these mechanisms depends on the kind of animals concerned and also on the relationship between the mating partners.

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The genus Peromyscus, the common deermouse, is divided into many species groups, species, and subspecies. As a rule, a limited number of intersubspecific and interspecific crosses may occur within each species group (Dice, 1933). Hence, the animals are suitable for a study of some of the mechanisms responsible for hybrid sterility. Liu (1953) and Clark and Liu (MS) have analyzed the fertility relationships of three forms of mice from the Peromyscus maniculatus species group. The criterion of fertility in these studies was the number of living young born per mated female. It was found that in the intersubspecific crosses fertility was of the same order of magnitude as that of both parent stocks, but in the interspecific crosses the average fertility was much reduced.

The differences observed in fertility in different crosses are necessarily the result of the action of one or more of the four mechanisms listed above. Blair and Howard (1944) have studied sexual isolation within a species group and have reported that sterility in hybridization parallels the sexual isolation between parent stocks. The importance of the other three mechanisms in Peromyscus remains to be investigated.

It is known that early prenatal mortality in mammals, due either to the failure of some of the liberated ova to be fertilized or to complete resorption of the zygotes or early embryos, is indicated by a discrepancy between the number of corpora lutea in the ovaries and the total number of embryos in the uterine horns. Later embryonic inviability is indicated by the presence of embryonic remnants (Brambell and Mills, 1948). The study of the relative importance of the various mechanisms involved in the partial sterility of mammalian hybridization may be reduced to the investigation of prenatal mortality, which is here defined as mortality occurring at any stage of the developing organism prior to birth, including the unfertilized ovum. The purpose of this paper is to present some results of a study of prenatal mortality.

Acknowledgments

I wish to express my appreciation to Dr. James V. Neel for his helpful advice and guidance throughout this investigation. Thanks are also due Dr. Lee R. Dice and Dr. William J. Schull for their valuable suggestions and criticisms in reading the manuscript. The photographs were taken by Mr. William B. McIntosh to whom I am greatly indebted. The Institute of Human Biology, University of Michigan, has provided the necessary equipment for this study and has been responsible for the care of the laboratory animals.

MATERIALS AND METHODS

All the mice used in this study belong to the Peromyscus maniculatus species group.

Peromyscus maniculatus bairdi, from Washtenaw County, Michigan. — Stock collected by Van T. Harris, 1946.

Peromyscus maniculatus blandus, from Tularosa, Otero County, New Mexico. — Stock collected by W. F. and F. A. Blair, 1938.

Peromyscus polionotus leucocephalus, from Santa Rosa Island, Florida. — Stock collected by W. Frank Blair, 1942.

The subspecies bairdi is restricted to a prairie habitat. It is relatively small and light-bodied, with a buff and gray coloration. The subspecies blandus is found in a desert habitat. It is comparatively large and heavy-bodied with color more vinaceous. The geographic ranges of these two subspecies do not meet, but the two are connected by geographically and morphologically intermediate forms. The species polionotus is geographically isolated from the species maniculatus by a gap of several hundred miles where neither species occurs (Osgood, 1909). Leucocephalus is considerably smaller than either bairdi or blandus. It is very pale and lives in beach dunes.

The animals utilized in the present investigations have been reared by the methods described by Dice (1947). For each test mating a pair of sexually mature mice was isolated in a cage for six months to one and one-half years, or until the female was sacrificed, whichever occurred first. Matings with the relationship closer than first cousin and those in which the age of the female was greater than 83 weeks were excluded, except as otherwise mentioned. All matings were examined for litters or pregnancies every day until the female was killed. Pregnant females were killed at a late stage of gestation, usually one or two days from their full term. With experience this stage of gestation can readily be determined by simple palpation of the abdomen. The number of corpora lutea in each ovary was counted under a binocular dissecting microscope. The uteri were soaked in tap water in order to reveal "moles" (partly resorbed embryos and placentas) more clearly and also to prevent the uteri and their contents from drying out. The relative position in the uterus of each embryo was recorded and the sexes were identified if they were normally developed.

In the descriptions of the intersubspecific and interspecific crosses the female parent is named first; in the F_1 x F_1 matings the various possible combinations are not considered separately but are treated as one, since they can not be shown to be different statistically in relation to prenatal mortality.

PRENATAL MORTALITY IN PARENT STOCKS

During pregnancy only one conspicuous set of corpora lutea is found in the ovary. This set is formed from the follicles which rupture at the ovulation preceding conception. Except in rare cases, when polyovulation or anovulation occurs, the corpora lutea of pregnancy offer the most reliable measure of ovarian activity (MacDowell, 1924). The numbers of corpora lutea and of normal fetuses in pregnant females of the three stocks, Peromyscus maniculatus bairdi, P. m. blandus, and P. poliotonus leucocephalus, are given in Table I and are illustrated in Figure 1.

Although the order of ovarian activity, as revealed by the average number of corpora lutea of pregnancy per female, is bairdi > blandus > leucocephalus, none of the differences between strains in this respect reaches the level of significance.

TABLE I

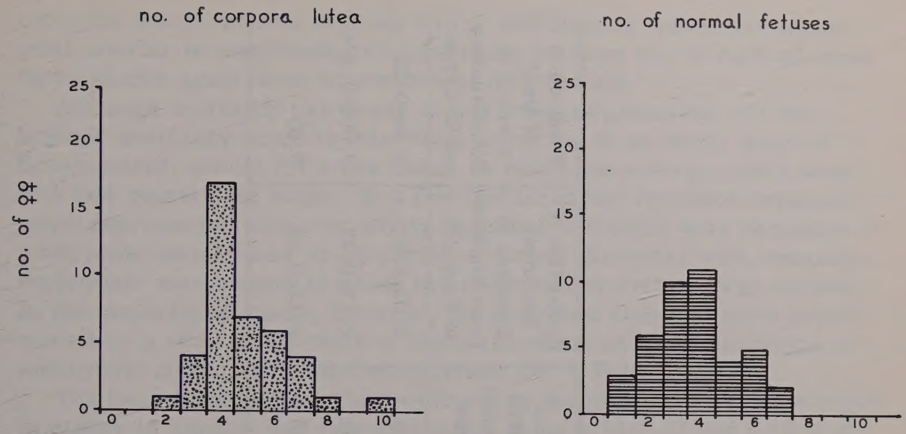
Number of Corpora Lutea and of Normal Fetuses in Three Parent Stocks

Stock	Number of ♀♀ Examined	Number of Corpora Lutea per Pregnant Female (Mean ± S. E.)	Number of Normal Fetuses per Pregnant Female (Mean ± S. E.)
P. m. bairdi	41	4.85±0.24	3.73±0.25
P. m. blandus	59	4.78±0.15	3.39±0.17
P. p. leucocephalus	5	4.60±0.24	2.80±0.49

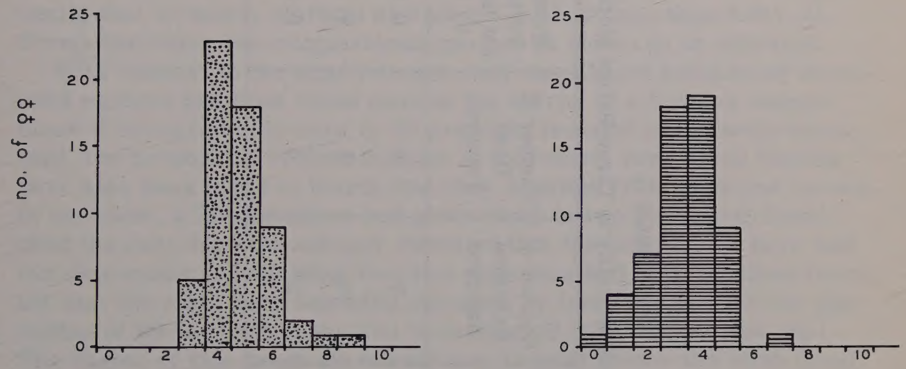
The number of normal fetuses per pregnant female shows the same trend. The value is greatest in bairdi, intermediate in blandus, and lowest in leucocephalus. Although the differences in the numbers of normal fetuses are greater than those in the numbers of corpora lutea, they still do not reach the level of significance.

The data of Table I show a higher number of corpora lutea than of normal fetuses. This discrepancy can only be explained by prenatal mortality. Prenatal mortality can be classified in three categories according to the developmental stage at which mortality occurs: (1) mortality occurring before or during implantation (corpora lutea not represented by embryos or embryonic remnants), (2) mortality occurring between a period shortly after implantation and approximately mid-term (partly resorbed embryos and placentas, which will be referred to henceforth as resorbed embryos), and (3) mortality occurring subsequent to mid-term (dead fetuses). The various values of these categories in the three parent stocks are summarized in Table II.

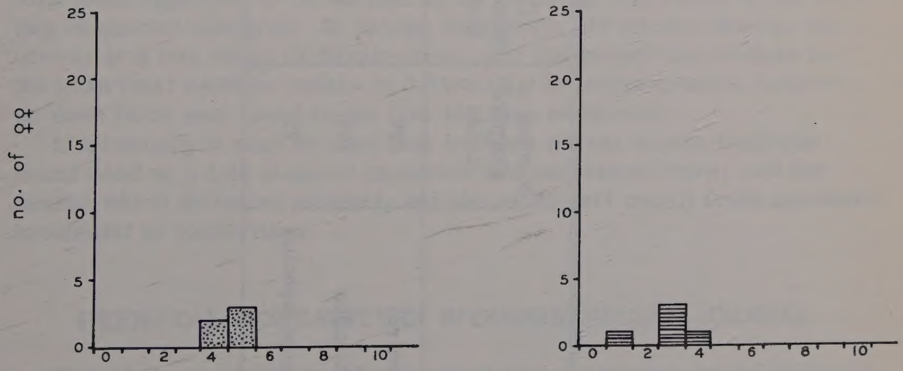
Mortality occurring before or during implantation may be due to any of the following causes: (1) liberated ova remain unfertilized; (2) ova are fertilized but fail to develop; or (3) ova are fertilized and develop up to approximately three days, then die and are completely resorbed. As no study of this early period of prenatal mortality was undertaken, the respective effects of these three causes were not distinguished. The category, "corpora lutea not represented by embryos," may therefore include any or all of these three factors. With respect to this



(a) P. m. bairdi



(b) P. m. blandus



(c) P. p. leucocephalus

Fig. 1. Number of corpora lutea and of normal fetuses in three parent stocks.

TABLE II

Prenatal Mortality in Three Parent Stocks

Stock	Number of Corpora Lutea	Total Number of Embryos	Number of Resorbed Embryos	Number of Dead Fetuses	Proportion of Corpora Lutea not Represented by Embryos	Proportion of Resorbed Embryos to Total Number of Embryos	Proportion of Dead Fetuses to Total Number of Embryos
P. m. bairdi	199	164	5	6	0.18±0.03	0.03±0.01	0.04±0.02
P. m. blandus	282	231	30	1	0.18±0.02	0.13±0.02	0.004±0.004
P. p. leucocephalus	23	16	2	0	0.30±0.10	0.13±0.08	0.00

category leucocephalus exceeds bairdi and blandus, which are somewhat similar in magnitude. Comparisons between two of each of these three stocks again show no statistical differences.

Although mortality can occur at any stage of gestation, all embryonic mortality noted in this study occurred at an early stage of development, except for a few cases in which the embryos were dead in a late gestational stage. In a few instances the resorbed embryos were represented either by partly resorbed placentas with remnants of embryonic membranes or by partly resorbed placentas with complete embryonic membranes in which the rudimentary embryo was located. In the majority of cases, however, the resorbed embryos were represented by a very small piece of tissue in which no placental tissue or embryonic tissue could be distinguished (Pl. I, Figs. 1 and 2).

The proportion of resorbed embryos to the total number of embryos is higher in blandus and leucocephalus, being 0.13±0.02 and 0.13±0.08, respectively, whereas in bairdi it is low, being only 0.03±0.01. Statistically, the proportion of resorbed embryos in blandus exceeds significantly that in bairdi (normal deviate $c = 4.47$, P less than 0.01), although the other two comparisons can not be shown to be different.

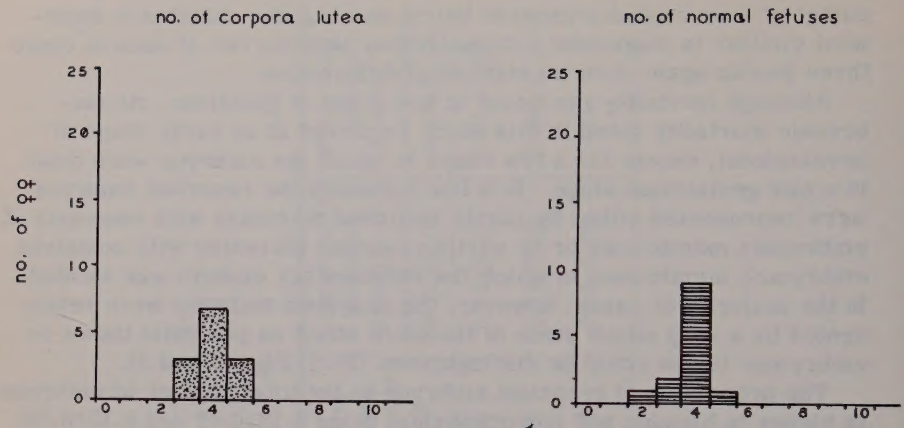
With respect to the dead fetuses, only one almost completely developed embryo has been found dead in the uterus of a blandus female. Since it occurred only once in 59 pregnant females which were examined, the proportion of dead fetuses is extremely low. Dead fetuses have also been found in bairdi, but they resulted from different causes. In one case, a female which had given birth to two young was found dead the next day. An autopsy revealed that the left uterine horn had two depressions, indicating that two placentas had been attached there, but that the right horn was still occupied by five fetuses. All the placentas of these five fetuses had been torn off from the uterine wall. The vagina of this pregnant female was twisted; hence the birth canal was blocked. In another instance one young had been born, and the female was found dead with another fetus retained in the uterus. Since this fetus appeared to be normal in all respects, the cause of the mishap remained unsolved. In bairdi, therefore, six fetuses died in the uterus at a late stage of development, and the proportion of dead fetuses to total embryo counts is 0.04±0.02. In leucocephalus, however, no dead fetus was found in the five females examined.

In summary it may be said that in these parent stocks embryos found dead at a late stage of gestation are extremely rare, and the deaths which do occur probably for the most part result from accidents incidental to parturition.

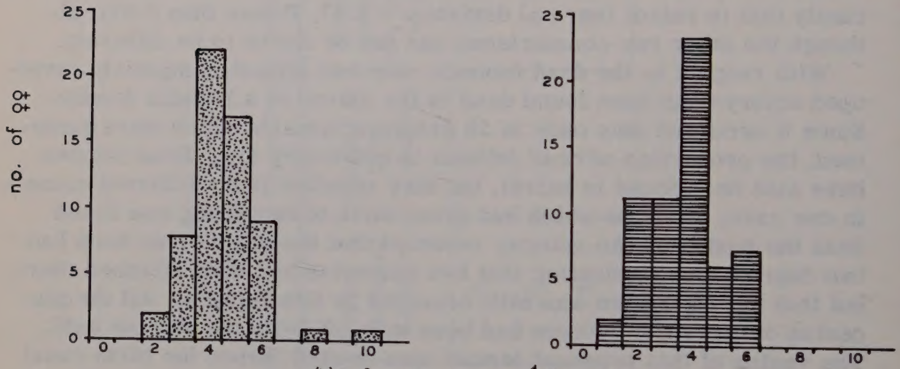
PRENATAL MORTALITY IN INTERSUBSPECIFIC CROSSES

The numbers of corpora lutea and of normal fetuses in the intersubspecific crosses between bairdi and blandus are given in Table III and are illustrated in Figure 2.

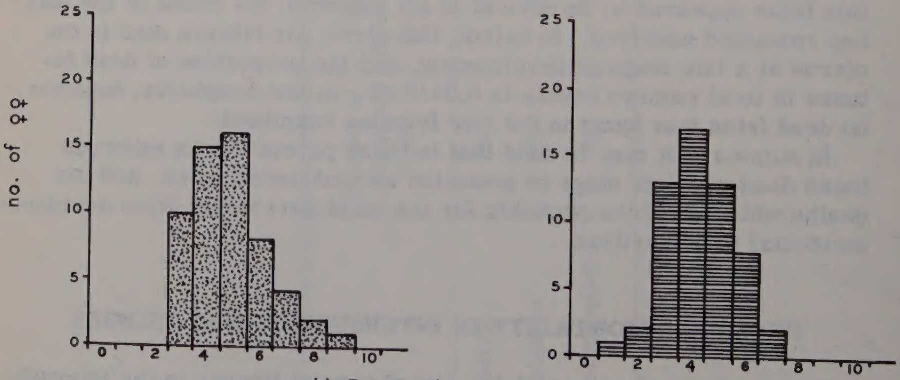
In the cross bairdi x blandus, the average number of corpora lutea



(a) ♀ P. m. bairdi x ♂ P. m. blandus



(b) ♀ P. m. blandus x ♂ P. m. bairdi



(c) F_1 x F_1 (bairdi x blandus)

Fig. 2. Number of corpora lutea and of normal fetuses in intersubspecific crosses.

found in each pregnant female is 4.00±0.20, which is lower than that in bairdi inter se, but does not differ statistically (t=1.91, P=0.10-0.05). In the reciprocal cross blandus x bairdi, the number of corpora lutea is 4.55±0.17, which is close to that of the female parent stock. The average number of corpora lutea found in the F_1 x F_1 mating is 4.84 ±0.19, which is intermediate between that in the two parent stocks. The hybrid females, therefore, have the same ovarian activity as their parent stocks insofar as this is indicated by the number of corpora lutea.

TABLE III

Number of Corpora Lutea and of Normal Fetuses in Intersubspecific Crosses Between P. m. bairdi and P. m. blandus

Cross	Number of ♀♀ Examined	Number of Corpora Lutea per Pregnant Female (Mean ± S. E.)	Number of Normal Fetuses per Pregnant Female (Mean ± S. E.)
bairdi x blandus	13	4.00±0.20	3.77±0.20
blandus x bairdi	60	4.55±0.17	3.68±0.17
F_1 x F_1 (bairdi x blandus)	56	4.84±0.19	4.27±0.17

The average number of normal fetuses found in both reciprocal crosses is very close to the mean value of the two parent stocks. In the F_1 x F_1 mating, however, this value is higher and exceeds that in the cross blandus x bairdi (t=2.43, P=0.02-0.01), although it can not be shown to be different from that in the cross bairdi x blandus (t=1.36, P=0.20-0.10).

Since the number of normal fetuses in the crosses between bairdi and blandus is either close to or higher than that of the parent stocks, the prenatal mortality is necessarily low. Various categories of prenatal mortality are shown in Table IV. It is clearly seen that in both the reciprocal crosses the proportion of corpora lutea not represented by embryos is lower than that in both parent stocks. Their values are 0.04±0.02 and 0.13±0.02, respectively. The F_1 x F_1 mating shows the same trend, the value being intermediate to the crosses between bairdi and blandus.

The proportion of resorbed embryos parallels the proportion of corpora lutea not represented by embryos. Their values in the bairdi x blandus cross and in the F_1 x F_1 mating are very low; they are 0.02 ±0.02 and 0.02±0.01, respectively. The value in the blandus x bairdi mating is a little higher, but still lower than that in blandus inter se.

Dead fetuses were found only in an expectant female from the cross blandus x bairdi. In this instance the pregnant female was found dead after two young had been born. At autopsy, besides one resorbed embryo, two fetuses were found in the uterus, and a third fetus had entered the vagina. All these phenomena showed clearly that parturition was in progress. The cause of death is uncertain, but to judge from the

TABLE IV

Prenatal Mortality in Intersubspecific Crosses Between P. m. bairdi and P. m. blandus

Cross	Number of Corpora Lutea	Total Number of Embryos	Number of Resorbed Embryos	Number of Dead Fetuses	Proportion of Corpora Lutea not Represented by Embryos	Proportion of Resorbed Embryos to Total Number of Embryos	Proportion of Dead Fetuses to Total Number of Embryos
bairdi x blandus	52	50	1	0	0.04±0.02	0.02±0.02	0.00
blandus x bairdi	273	238	17	3	0.13±0.02	0.07±0.02	0.01±0.01
F_1 x F_1 (bairdi x blandus)	271	242	4	0	0.11±0.02	0.02±0.01	0.00

relatively large size of the fetuses, fetal-pelvic disproportion may have been a factor.

PRENATAL MORTALITY IN INTERSPECIFIC CROSSES

Since leucocephalus and blandus are assigned to different species, crosses between them are particularly interesting from the viewpoint of prenatal mortality. The numbers of corpora lutea and of normal fetuses are shown in Table V and are illustrated in Figure 3.

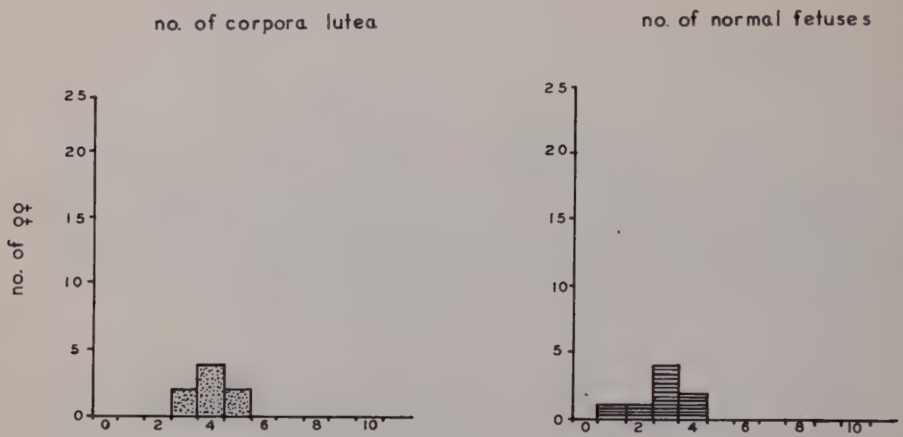
TABLE V

Number of Corpora Lutea and of Normal Fetuses in Interspecific Crosses Between P. m. blandus and P. p. leucocephalus

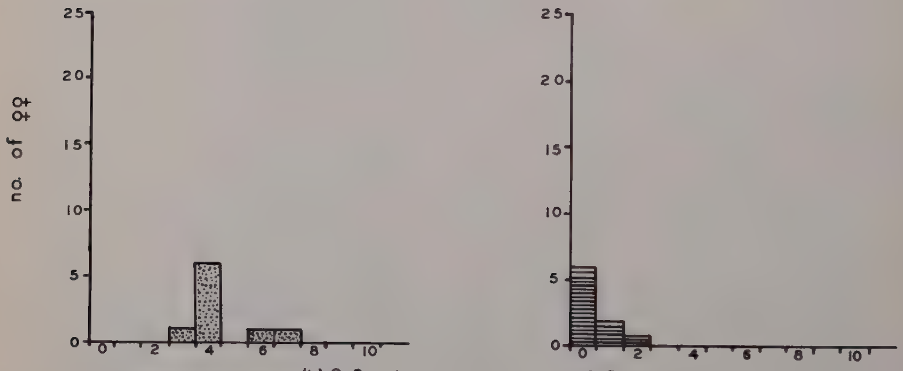
Cross	Number of ♀♀ Examined	Number of Corpora Lutea per Pregnant Female (Mean ± S. E.)	Number of Normal Fetuses per Pregnant Female (Mean ± S. E.)
blandus x leucocephalus	8	4.00±0.27	2.88±0.36
leucocephalus x blandus	9	4.44±0.13	0.44±0.08
F_1 x F_1 (blandus x leucocephalus)	22	4.55±0.21	3.32±0.24

The number of corpora lutea per pregnant female in the blandus x leucocephalus mating is 4.00±0.27, which is slightly lower than that in blandus mated inter se. As would be expected, the small discrepancy is probably due to sampling error ($t=1.80$, $P=0.10-0.05$). Since ovulation occurs spontaneously during estrum (Clark, 1936), there is no reason to suspect that the number of corpora lutea per pregnant female could be affected by the male stock used. The corpora lutea per pregnancy in leucocephalus x blandus is 4.44±0.13, which is close to that in leucocephalus mated inter se. In the F_1 x F_1 mating the value is 4.55 ±0.21, which is very close to the value of both parent stocks.

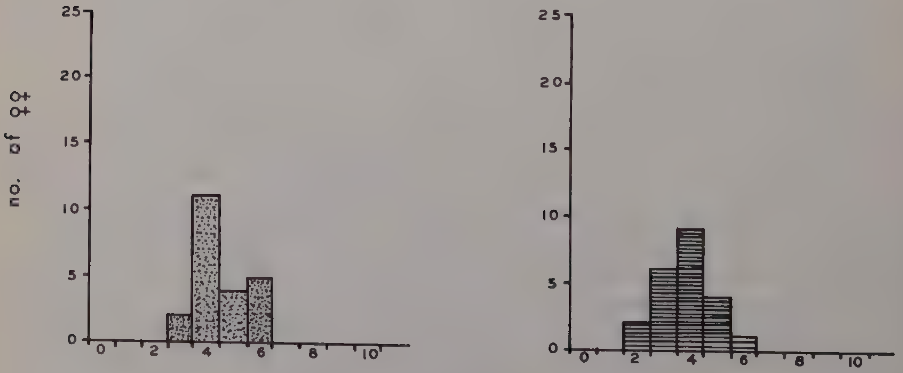
In the cross blandus x leucocephalus and its reciprocal the number of normal fetuses found per pregnancy is quite different, being 2.88±0.36 and 0.44±0.08, respectively. Because of this fact and because of a similar although less apparent difference in the intersubspecific crosses between bairdi and blandus, it is likely that the female parent has a more profound influence on prenatal mortality than has the male parent. The average number of normal fetuses in the cross blandus x leucocephalus falls more or less into the same magnitude as the values observed in both parent stocks, but the number in the leucocephalus x blandus cross is much lower and differs statistically from that of both parent stocks, blandus and leucocephalus ($t=6.74$ and $t=4.88$, P less than 0.01). The number of normal fetuses in the F_1 x F_1 mating is 3.32±0.24, which exceeds that in both parent stocks and in the F_1 hybrids.



(a) ♀ P. m. blandus × ♂ P. p. leucocephalus



(b) ♀ P. p. leucocephalus × ♂ P. m. blandus



(c) F_1 × F_1 (blandus × leucocephalus)

Fig. 3. Number of corpora lutea and of normal fetuses in interspecific crosses.

The cause of the wide difference in the numbers of normal fetuses in the reciprocal crosses is analyzed in Table VI. The proportions of corpora lutea not represented by embryos in the crosses blandus x leucocephalus and F_1 x F_1 (blandus x leucocephalus) are the same and are very close to those of the parent stocks. The proportions of resorbed embryos are also very similar in these two crosses and they resemble those of their parent stocks. Furthermore, dead fetuses were not discovered in these two crosses. On the other hand, the cross leucocephalus x blandus falls into an entirely different category. In this cross the proportion of corpora lutea not represented by embryos is very much higher (0.47±0.08). It is different statistically from that in the cross blandus x leucocephalus and in F_1 x F_1 (the normal deviates $c = 3.10$ and $c = 3.47$, P being less than 0.01).

In addition to the high proportion of corpora lutea not represented by embryos, the cross leucocephalus x blandus resulted in a high proportion of dead fetuses. In one instance a female died one day after giving birth to a single offspring. At autopsy one abnormally large fetus was found in the vagina. It is thought that the disproportionally large size of the fetus may have been the cause of death. In five out of nine fertile matings the mice died close to term from a different cause. One or two days prior to death a viscous, hemorrhagic discharge appeared in the vaginal outlet. The mice moved very slowly and were not responsive to stimuli which usually evoke considerable activity. At autopsy serosanguineous fluid filled the cavity between the amniotic sacs and the uterine wall. Sometimes only one of the uterine horns was involved. Fetuses were usually two or three days from full term and appeared perfectly normal insofar as the external and internal morphology was concerned.

A few observations, made late in the course of the investigation, suggest that the size of placentas of the dead mice from the cross leucocephalus x blandus was greater than the size of the placentas of the normal mice from the cross blandus x leucocephalus (Pl. I, Figs. 3 and 4). Hence several measurements have been taken, the results of which are given in Table VII.

The mean value of the longest diameter of the placentas in the leucocephalus x blandus cross is 1.93 cm, which is statistically larger than the value in the blandus x leucocephalus cross, which is only 1.07 cm. The difference observed is not thought to be the result of postmortem changes. Because of this fact and also because of intra-uterine bleeding, it can be postulated that a placental overgrowth resulted in a disharmonious relationship between placental and maternal tissues. Further observations on this point are highly desirable.

PRENATAL MORTALITY AND MATERNAL AGE

There is an impression that when female mice reach a relatively advanced age the average litter size decreases. Since the genetic constitution of the female remains unchanged, one would expect either the

TABLE VI

TABLE VI

Prenatal Mortality in Interspecific Crosses
Between P. m. blandus and P. p. leucocephalus

Cross	Number of Corpora Lutea	Total Number of Embryos	Number of Resorbed Embryos	Number of Dead Fetuses	Proportion of Corpora Lutea not Represented by Embryos	Proportion of Resorbed Embryos to Total Number of Embryos	Proportion of of Dead Fetuses to Total Number of Embryos
blandus x leucocephalus	32	27	4	0	0.16±0.06	0.15±0.07	0.00
leucocephalus x blandus	40	21	0	17	0.47±0.08	0.00	0.81±0.09
F_1 x F_1 (blandus x leucocephalus)	100	84	11	0	0.16±0.04	0.13±0.04	0.00

number of ova liberated per estrous cycle to decrease or the prenatal mortality to increase with advance in maternal age.

In the two parent stocks, bairdi and blandus, the age of females examined ranged from a few months to more than two years. Hence, these mice were suitable for the present analysis. They have been arbitrarily divided into two age groups: (1) "prime" age, from sexual maturity to the age of 82 weeks (a little over one and one-half years old), and (2) "advanced" age, 83 weeks or older. Mice of the former group were used in the previous analysis, but mice of the latter group were not used. The data derived from the latter group are available for the analysis of maternal effect at an advanced age.

In this analysis the first step has been to determine whether or not the litter size actually decreases when the female mice reach an advanced age. In the preceding pages it has been shown that most prenatal mortality occurs at an early stage of development. Usually, the average litter size in mice is evaluated from the number of living young shortly after birth, but even by that time the young mice have been exposed to an early stage of postnatal mortality. The number of

TABLE VII

Length of the Longest Diameter of Placentas in Interspecific Crosses Between P. m. blandus and P. p. leucocephalus

Cross	Number of Placentas Measured	Mean Longest Diameter (cm)	t	P
leucocephalus x blandus	4	1.93	10.82	< 0.01
blandus x leucocephalus	6	1.07		

TABLE VIII

Relation of Litter Size to Two Age Groups in Two Parent Stocks, P. m. bairdi and P. m. blandus

All female mice were sacrificed one or two days before parturition.

Cross	Age Group	Number of ♀♀ Examined	Average Litter Size (Based on the Number of Normal Fetuses)
bairdi	Prime age	41	3.73
	Advanced age	11	3.45
blandus	Prime age	59	3.39
	Advanced age	21	2.05

normal late fetuses, which have survived the early stage of prenatal mortality and have not yet been exposed to any postnatal mortality, appears, therefore, to be the most reliable source for the estimation of average litter size. The values thus obtained and their statistical treatment (Brandt, 1933) are shown in Tables VIII and IX.

TABLE IX

Analysis of Variance for Litter Size in Relation to Age Groups in P. m. bairdi and P. m. blandus

Source	d.f.	Sum of Square	Mean Square	F	$F_{.05}$	$F_{.01}$
Stocks	1	17.12	17.12	9.56	....	6.84
Ages	1	22.34	22.34	12.48	....	6.84
Interaction (ages x stocks)	1	1.84	1.84	1.03	3.92	....
Error (between individual females)	128	229.76	1.79	....	....	....
Total	131	271.06				

Table IX, besides indicating that the litter size in bairdi exceeds that in blandus regardless of age, shows clearly that the litter size decreases with advancing age (F = 12.48, significant at the 1 per cent level). Tables X, XI, and XII contain an analysis of the factors responsible for this finding.

TABLE X

Relation of Corpora Lutea Counts to Two Age Groups in Two Parent Stocks, P. m. bairdi and P. m. blandus

Cross	Age Group	Number of ♀♀ Examined	Average Number of Corpora Lutea
bairdi	Prime age	41	4.85
	Advanced age	11	5.73
blandus	Prime age	59	4.78
	Advanced age	21	4.19

Table X shows that the corpora lutea counts do not differ significantly in relation to the two age groups. On the other hand, Table XII shows that when the females are of advanced age both bairdi and blandus have a higher proportion of corpora lutea which are not represented by embryos. The values for the advanced age in bairdi and blandus are 0.29 and 0.34, respectively, and the values for the prime age are lower, both 0.18. The proportion of resorbed embryos to total corpora lutea shows the same trend. In bairdi the proportion of resorbed embryos in the advanced age is 0.11, and that in the prime age is only 0.03. In blandus the proportion of resorbed embryos in the advanced age is 0.17, and that in the prime age is 0.11. The chi-square test shows that these figures are statistically different. Finally, the heterogeneity chi-square is found to be very low. The two sets of data, one from bairdi and the other from blandus, thus agree. By the method of partition of chi-square one may test which of these two factors, namely, corpora lutea not represented by embryos, and resorbed embryos, is more responsible for the higher prenatal mortality in advanced age. Tables XIII and XIV show that both the items "normal fetuses vs. corpora lutea not represented by embryos" and "normal fetuses vs. resorbed embryos" are significantly different in two age groups in bairdi and in blandus. It may be said, therefore, that when the female mice reach an advanced age, the average number of normal fetuses per pregnancy (or average litter size) decreases. This decrease is apparently not due to the release of fewer eggs at ovulation, as judged by the number of corpora lutea, but is a consequence both of a higher proportion of corpora lutea not being represented by embryos and of a higher proportion of those which develop embryos being partly resorbed.

TABLE XI

Analysis of Variance for Corpora Lutea Counts in Relation to Age Groups in P. m. bairdi and P. m. blandus

Source	d.f.	Sum of Square	Mean Square	F	$F_{.05}$	$F_{.01}$
Stocks	1	17.18	17.18	8.42	....	6.84
Ages	1	0.10	0.10	0.05	3.92	....
Interaction (ages x stocks)	1	0.10	0.10	0.05	3.92	....
Error (between individual females)	128	260.68	2.04	....	....	....
Total	131	278.06				

PRENATAL MORTALITY AND INBREEDING

It has been shown that prenatal mortality in three stocks of Peromyscus differs in extent, although statistically significant differences

TABLE XII

Maternal Age in Relation to Prenatal Mortality in P. m. bairdi and P. m. blandus

Cross	Age Group	Number of ♀♀ Examined	Corpora Lutea not Represented by Embryos		Resorbed Embryos		Normal Fetuses		Total	χ^2	P
			Number	Proportion	Number	Proportion	Number	Proportion			
bairdi	Prime	41	35	0.18	5	0.03	153	0.79	193 } 256	12.14	<0.01
	Advanced	11	18	0.29	7	0.11	38	0.60	63		
blandus	Prime	59	51	0.18	30	0.11	200	0.71	281 } 369	15.05	<0.01
	Advanced	21	30	0.34	15	0.17	43	0.49	88		
Total	Prime	100	86	0.18	35	0.07	353	0.75	474 } 625	23.58	<0.01
	Advanced	32	48	0.32	22	0.14	81	0.54	151		

Analysis of Chi-square

Item	χ^2	d. f.	P
Total	27.19	4	<9.01
Deviation	23.58	2	<0.01
Heterogeneity	3.61	2	0.20-0.10

have not been demonstrated. That genetic factors, such as recessive lethal and semilethal genes, are partly responsible for the observed differences in prenatal mortality in various crosses is a possibility that may be explored. If such factors are operative, embryos resulting from matings of closely related individuals will have higher mortality than embryos from matings of less closely related or of unrelated individuals, because in the former these genes are more likely to occur in the homozygous condition causing death.

TABLE XIII

Partition of Chi-square of Table XII for Prenatal Mortality in Relation to Age Groups in P. m. bairdi

Source	χ^2	d.f.	P
Normal fetuses vs. resorbed embryos	8.99	1	<0.01
Normal fetuses + resorbed embryos vs. corpora lutea not represented by embryos	3.15	1	0.10-0.05
Total	12.14	2	<0.01

Source	χ^2	d.f.	P
Normal fetuses vs. corpora lutea not represented by embryos	4.42	1	0.05-0.02
Normal fetuses + corpora lutea not represented by embryos vs. resorbed embryos	7.72	1	<0.01
Total	12.14	2	<0.01

In the crosses blandus inter se, F_1 x F_1 (blandus x bairdi) and F_1 x F_1 (blandus x leucocephalus), data have been accumulated on the results of brother-sister matings in addition to the data on ordinary matings used in all previous analyses. Hence, each cross consists of two sets of data with respect to the kinds of relationships, namely, one set of data from the matings where the relationship of the partners was not closer than first cousin, as in blandus inter se, or where the partners were unrelated as in F_1 x F_1 (blandus x bairdi) and F_1 x F_1 (blandus x leucocephalus), and the other set of data from the matings where the relationship of partners was that of full sibs, although they may have belonged to different litters. Since it is desirable to make the maternal age as uniform as possible to reduce the maternal effect, only the results of the first pregnancy are used in the analysis.

For convenience of statistical treatment, the kinds of prenatal mortality, i.e., proportion of corpora lutea not represented by embryos and the proportion of resorbed embryos to total number of embryos, as well as their chi-square values, are shown separately in Tables XV and XVI.

TABLE XIV

Partition of Chi-square of Table XII for Prenatal Mortality in Relation to Age Groups in P. m. blandus

Source	χ^2	d.f.	P
Normal fetuses vs. resorbed embryos	5.11	1	0.05-0.02
Normal fetuses + resorbed embryos vs. corpora lutea not represented by embryos	9.94	1	<0.01
Total	15.05	2	<0.01

Source	χ^2	d.f.	P
Normal fetuses vs. corpora lutea not represented by embryos	12.51	1	<0.01
Normal fetuses + corpora lutea not represented by embryos vs. resorbed embryos	2.54	1	0.20-0.10
Total	15.05	2	<0.01

It is seen from Table XV that in matings of blandus inter se and of F_1 x F_1 (blandus x leucocephalus) the number of corpora lutea not represented by embryos is higher in brother-sister matings, but conversely in the cross F_1 x F_1 (blandus x bairdi) the number is higher in the unrelated matings. Hence, the results of different crosses are contradictory, although the chi-square tests show that these values are not significantly different.

TABLE XV

Proportion of Corpora Lutea not Represented by Embryos in Relation to Degree of Inbreeding

Cross	Relationship Between Mating Partners	Corpora Lutea not Represented by Embryos		Corpora Lutea Represented by Embryos		Total	χ^2	P
		Number	Proportion	Number	Proportion			
blandus inter se	Brother-sister	10	0.19	44	0.81	54	0.87	0.50-0.30
	Not closely related	15	0.13	100	0.87	115		
F_1 x F_1 (bairdi x blandus)	Brother-sister	21	0.15	122	0.85	143	1.00	0.50-0.30
	Unrelated	25	0.19	105	0.81	130		
F_1 x F_1 (blandus x leucocephalus)	Brother-sister	5	0.33	10	0.67	15	0.81	0.50-0.30
	Unrelated	7	0.21	26	0.79	33		

TABLE XVI

Proportion of Resorbed Embryos in Relation to Degree of Inbreeding

Cross	Relationship Between Mating Partners	Resorbed Embryos		Normal Embryos		Total	χ^2	P
		Number	Proportion	Number	Proportion			
blandus inter se	Brother-sister	5	0.11	39	0.89	44	0.76	0.50-0.30
	Not closely related	7	0.07	93	0.93	100		
F_1 x F_1 (bairdi x blandus)	Brother-sister	13	0.11	109	0.89	122	5.24	0.05-0.02
	Unrelated	3	0.03	102	0.97	105		
F_1 x F_1 (blandus x leucocephalus)	Brother-sister	2	0.20	8	0.80	10	0.43	0.70-0.50
	Unrelated	3	0.12	23	0.88	26		
Total	Brother-sister	20	0.11	156	0.89	176	4.41	0.05-0.02
	Unrelated or not closely related	13	0.06	218	0.94	231		

Analysis of Chi-square

Item	χ^2	d. f.	P
Total	6.43	3	0.10-0.05
Deviation	4.41	1	0.05-0.02
Heterogeneity	2.02	2	0.30-0.50

On the other hand, Table XVI shows that the proportion of resorbed embryos to total number of embryos is higher in the brother-sister matings. The values of resorbed embryos are 0.11 in blandus, 0.11 in F_1 x F_1 (blandus x bairdi), and 0.20 in F_1 x F_1 (blandus x leucocephalus). In matings of blandus inter se and of F_1 x F_1 (blandus x leucocephalus), however, the sample sizes are not large enough to make the values in brother-sister matings differ statistically from those values in the matings which are not closely related. In F_1 x F_1 (blandus x bairdi) matings, the proportion of resorbed embryos in the brother-sister matings does differ statistically at the 5 per cent level ($\chi^2 = 5.24$) from that in the unrelated matings. The total chi-square turns out to be 4.41, which is significant at the 5 per cent level for one degree of freedom. As would be expected, the heterogeneity test shows that data on the proportion of resorbed embryos in these crosses are in agreement with each other ($\chi^2 = 2.02$, P=0.50-0.30).

The analysis given above indicates that close inbreeding probably has no significant effect on the proportion of corpora lutea not represented by embryos, but that it increases the number of resorbed embryos.

RESORBED EMBRYOS AND SEXES

There is an indication that in some animals, especially man, the frequency of abortive embryos is higher among male embryos than among female embryos. One of the several hypotheses by which an attempt is made to explain such a phenomenon states that sex-linked lethal and semilethal genes are responsible for the sex-differential mortality (Little and Gibbons, 1921, cited from Spuhler, 1948). In Peromyscus it has been shown that genetic factors play a role in the occurrence of resorbed embryos. It would then be interesting to know whether the occurrence of resorbed embryos is due mainly to autosomal lethal and semilethal genes, or whether sex-linked genes take an active part also. To demonstrate this it is necessary to divide each type of mating into two classes, one with and the other without resorbed embryos, and to analyze the difference in sex ratio with respect to these two classes. The data have been compiled for this purpose and are summarized in Table XVII.

In all of the crosses, except in bairdi x blandus and F_1 x F_1 (blandus x bairdi), the pregnancies in which there occurs one or more resorbed embryos show a higher number of males per female than do the pregnancies without resorbed embryos. For example, the value in the cross blandus x leucocephalus is 2.00 and that in the cross bairdi inter se is 1.62, whereas the respective values for the pregnancies without resorbed embryos are 0.47 and 0.85, although none of them shows a significant difference. The heterogeneity chi-square test indicates that the whole set of data is rather consistent. It may therefore be stated that sex-linked lethal and semilethal genes are not responsible for the occurrence of resorbed embryos in various crosses to any noticeable extent.

TABLE XVII

Proportion of Resorbed Embryos in Relation to Sex

Whenever the number of entries in a given class is four or less, either Yates' correction has been applied or the exact probability has been calculated (Fisher, 1948). The values thus obtained are put in parentheses.

Cross	Resorbed Embryos	Sex		Total	Number of Males per Female	χ^2	P
		Female	Male				
blandus	With	47	53	100	1.13	0.57	0.50-0.30
	Without	87	81	168	0.93		
bairdi	With	13	21	34	1.62	2.79	0.10-0.05
	Without	78	66	144	0.85		
leucocephalus	With	3	4	7	1.33	1.17	0.30-0.20
	Without	5	2	7	0.40	(0.29)	
bairdi x blandus	With	2	2	4	1.00	0.09	0.90-0.80
	Without	19	26	45	1.37	(0.05)	
blandus x bairdi	With	18	24	42	1.33	0.25	0.70-0.50
	Without	83	93	176	1.12		
blandus x leucocephalus	With	3	6	9	2.00	3.19	0.10-0.05
	Without	15	7	22	0.47	(1.92)	

leucocephalus x blandus	With	. . .	. . .	. . .	. . .	. . .	. . . .
	Without	. . .	. . .	. . .	. . .	. . .	. . . .
F_1 x F_1 (blandus x bairdi)	With	27	20	47	0.74	0.64	0.50-0.30
	Without	151	144	295	0.95		
F_1 x F_1 (blandus x leucocephalus)	With	15	15	30	1.00	0.60	0.50-0.30
	Without	30	21	51	0.70		
Total	With	128	145	273	1.13	1.82	0.20-0.10
	Without	468	440	908	0.94		

Analysis of Chi-square

Item	χ^2	d. f.	P.
Total	9.30	7	0.30-0.20
Deviation	1.82	1	0.20-0.10
Heterogeneity	7.48	6	0.30-0.20

DISCUSSION

The presence of resorbed or dead embryos in the uteri is direct evidence of prenatal mortality; however, the estimation of prenatal mortality from the discrepancy between the number of corpora lutea and the total number of embryos is subject to certain criticisms. As Hall and Davis (1950) have pointed out, some of the structures counted as corpora lutea may be Graafian follicles which do not release an ovum. In such cases the corpora lutea count is likely to be higher than the actual number of ova released. On the other hand, some follicles give rise to more than one ovum, that is, polyovulation occurs, and thus the intra-uterine mortality appears lower than it actually is. But in observation on serial sections of twenty-two ovaries of the parent stocks and of the hybrids in Peromyscus, neither Graafian follicles with more than one ovum nor luteinized follicles with trapped ova have been found. This appears to indicate that polyovular follicles and anovular luteinized follicles are comparatively rare, if present at all, in the females of this genus. The corpora lutea which are not represented by embryos, therefore, probably give a rather reliable estimation of the number of unfertilized ova and of completely resorbed embryos.

As the preceding analysis has shown, several factors influence prenatal mortality. Before conclusions can be reached concerning the amount of prenatal mortality in certain specific and subspecific crosses, it is necessary to demonstrate clearly that the observed differences are not due to variables, such as age or inbreeding, but are correlated with the taxonomic entities which are of particular concern in this paper.

The present observations reveal that when the mouse reaches a relatively advanced age, the average number of embryos per pregnancy decreases, both because of the higher proportion of corpora lutea which fail to be represented by embryos and also because a higher proportion of the developing embryos are partly resorbed. In this analysis it was not possible to hold the age of the male parents constant, but since it usually paralleled the age of the female parents, the decreased male fertility may be somewhat responsible for the increased proportion of corpora lutea not represented by embryos (Yerushalmy, 1939). It is apparent, however, that maternal effects are more important in this respect. Blandau and Jordan (1941) found a considerable increase in prenatal mortality in rats, particularly preimplantation loss, when fertilization was delayed a few hours. Whether delayed fertilization is a factor to be considered in natural matings is not known. Perhaps it may be encountered more often in old females. In addition, the intra-uterine environment may be less favorable for implantation and subsequent development in females of relatively advanced age. Hence, the embryos are more likely to die and to be resorbed. The validity of these suggestions can only be determined by further observations.

Genetic factors, either lethal or semilethal, are usually considered among the important causes of prenatal mortality in mammals. Corner (1923) believes that much of the prenatal mortality in the pig is due to genetic defects. He did not, however, have adequate genetic data to prove his statement. Hammond (1928, cited from Brambell, 1944) has reported that in an inbred strain of rabbits possibly several recessive genes operating through the mother are responsible for the fetal atrophy. But Hollander and Strong (1950) stated that in the house mouse (and probably also in the rat) the segregation of recessive lethal genes can not be an adequate genetic explanation of most of the mortality. On that basis one would expect very little mortality in the hybrid embryos of the crosses between different strains, whereas only a slight reduction in prenatal mortality in such crosses has been observed. From an analysis of the connection between mortality and closeness of relationship of mating partners, it has been clearly shown in Peromyscus that in brother-sister matings there is a general tendency to an increase in the mortality rate, especially among F_1 x F_1 (bairdi x blandus) hybrids. Since in the brother-sister mating the probability that the offspring will inherit the same semilethal or lethal genes from the female or male parent increases, a higher proportion of zygotes becomes homozygous for these recessive lethal genes, and consequently the number of resorbed embryos increases. The present observations, therefore, give strong evidence that genetic factors are partly responsible for the occurrence of prenatal mortality.

Evidence of sex-linked inheritance in rodents has thus far been limited to a few incompletely documented instances, mainly in house mice. This fact, plus cytological observations, has led White (1945) to the conclusion that the sex chromosome of rodents is composed mostly of heterochromatin and probably plays little part in the genetic control of morphogenetic processes, other than the determination of sex. Recently Hauschka, Goodwin, and Brown (1951) reported a sex-linked lethal gene in the house mouse. Falconer (1952) also has shown that the "tabby" in the house mouse is sex linked. Thus far no single sex-linked gene has been found in Peromyscus. Hence such genes, if they are present at all, would be very rare in Peromyscus. In the analysis of the relationship between resorbed embryos and sex ratio in Peromyscus, the pregnancies with resorbed embryos show an even higher proportion of males in comparison with the pregnancies without resorbed embryos, but all of the occurrences are not statistically significant. Hence, it may be said that sex-linked lethal and semilethal genes do not have any detectable effect on the prenatal mortality.

Thus far several possible causes of prenatal mortality have been discussed. It has been shown that increasing maternal age and close genetic relationship between mating partners tend to augment the incidence of prenatal mortality. Since all the matings used in the present study, except those used for particular purposes, such as to analyze the amount of inbreeding in relation to prenatal mortality, are of comparable age (i.e., all of prime age) and with relationship not closer than first cousin, these factors do not enter into the interpretation of the differences in prenatal mortality in various crosses.

The proportion of corpora lutea not represented by embryos and the proportion of resorbed embryos are low in the intersubspecific cross bairdi x blandus and a little higher in the reciprocal cross. In general, the mortality is comparable to that of parent stocks, as is also the mortality of the F_1 x F_1 mating. According to Osgood (1909), these two geographically isolated subspecies are connected by interbreeding forms. In the laboratory the fertility between these two subspecies is close to that existing in their parent stocks when no choice of mates is allowed. All these observations are compatible and indicate that the low prenatal mortality in these intersubspecific crosses is responsible for the high fertility between the two parent stocks.

In the interspecific crosses between blandus and leucocephalus the two reciprocal crosses show different tendencies with respect to prenatal mortality. In the cross blandus x leucocephalus the proportion of corpora lutea not represented by embryos and the proportion of resorbed embryos are close to those of the parent stocks, while in the cross leucocephalus x blandus, the situation is entirely different. The proportion of dead fetuses and the proportion of corpora lutea not represented by embryos are very high, but not one resorbed embryo has been found. The high proportion of dead fetuses is apparently due to increased inviability of hybrid zygotes, which may trace back to the disharmonious relationships between developing embryos and intrauterine environment, as has been pointed out. Although the mechanisms responsible for this "disharmony" are unknown, one obvious possibility involves a serological incompatibility, such as that illustrated by the Rh situation in man. Suppose that blandus is homozygous for a pair of dominant genes which are responsible for the production of an antigen, whereas leucocephalus lacks this gene. Then in the cross leucocephalus x blandus a recessive female would carry heterozygous fetuses and the antigen produced by the latter could penetrate the placenta, reach the body of a pregnant female, and cause the production of an antibody. When the antibody finds its way, again through the placenta, to the heterozygous fetuses, an immunological reaction occurs, resulting in the premature death of fetuses. It is easily seen that such reaction would not occur if blandus is used as the female parent. In order to determine the validity of this hypothesis, serological examination has been undertaken in two pregnant leucocephalus females which had been mated with blandus males. The serum obtained from these two leucocephalus mice failed to agglutinate the erythrocytes from blandus mice. Nevertheless, it is worth while to point out that if the hypothesis outlined above is the main factor responsible for the occurrence of dead fetuses in the cross leucocephalus x blandus, the same phenomenon should occur in some of the F_2 x F_2 matings because this particular gene pair would segregate in F_2 generation. With respect to this point, further experimental observations are highly desirable.

The high proportion of corpora lutea not represented by embryos is also worth further consideration. If it is assumed that most of the ova in the pregnant females from the cross leucocephalus x blandus were fertilized and began development but were completely resorbed later, some resorbed embryos should have been recovered. But since this was not the case, it is quite possible that a high proportion of liberated ova failed to be fertilized.

Two possible explanations of these findings deserve mention: (1) Since leucocephalus is considerably smaller in body size than blandus, mechanical isolation or physical incompatibility may have been responsible for a smaller quantity of spermatozoa being injected into the vagina. This, of course, could decrease the probability that a liberated egg would be fertilized. (2) Spermatozoa may have been injected into the cervix of the uterus but failed either to reach or to penetrate the ova. Either one or both of these factors can cause the failure of a larger proportion of corpora lutea to be represented by embryos.

Since leucocephalus and blandus are geographically isolated by a gap of several hundred miles where no intermediate forms exist, there is no opportunity for gene exchange between them in nature, genetic differences presumably have been accumulated, and gradually the sexual isolation between these two species has been developed (Blair and Howard, 1944). Even in the laboratory the fertility between the members of this particular cross, leucocephalus x blandus, is very low when no choice of mates is allowed, although in the reciprocal cross the fertility is higher (Liu, 1953; Clark and Liu, MS, Watson, 1942). These findings are fairly consistent with those of prenatal mortality, that is, increased prenatal mortality parallels high sterility. In other words, in the cross blandus x leucocephalus the fertility is comparable to that of the parent stocks: the proportion of corpora lutea not represented by embryos and the proportion of resorbed embryos are of the same magnitude as that of their parent stocks, but in the cross leucocephalus x blandus, the fertility is much reduced, a high proportion of liberated ova fails to be fertilized, and the viability of hybrid zygotes is considerably lower. It may be concluded, therefore, that, in addition to the existence of sexual isolation, the higher prenatal mortality of the crosses between blandus and leucocephalus substantiates the validity of assigning these two forms as separate species.

SUMMARY

1. Prenatal mortality has been studied with respect to three forms of the Peromyscus maniculatus species group and the two possible crosses between these forms.

2. From the examination of the ovaries and the uterine contents of a near-term, pregnant mouse, the stage of development at which prenatal death occurred can be estimated as follows: (a) mortality occurring before or during implantation (corpora lutea not represented by embryos or embryonic remnants), (b) mortality occurring between a period shortly after implantation and approximately mid-term (partly resorbed embryos and placentas), and (c) mortality occurring subsequent to mid-term (dead fetuses).

3. Prenatal mortality is not significantly different for P. maniculatus blandus, P. m. bairdi, and P. polionotus leucocephalus.

4. Fertility in the intersubspecific crosses between P. m. bairdi and P. m. blandus is comparable to that of the parent stocks, and the

proportion of corpora lutea not represented by embryos and the proportion of resorbed embryos are low.

5. In the interspecific crosses between blandus and leucocephalus the average fertility is reduced. This is due both to the fact that fewer liberated ova are fertilized in the pregnant female and to the fact that the proportion of dead fetuses is increased.

6. Prenatal mortality increases with the advance of maternal age.

7. Close inbreeding tends to augment prenatal mortality. This observation may be accounted for by the presence of autosomal lethal and semilethal genes. Inasmuch as no excess mortality was observed among male embryos, sex-linked lethal genes do not appear to play a significant role.

8. The parallelism between prenatal mortality and reduced fertility has been emphasized.

LITERATURE CITED

BLAIR, W. FRANK, and WALTER E. HOWARD

1944 Experimental evidence of sexual isolation between three forms of mice of the cenospecies Peromyscus maniculatus. Contrib. Lab. Vert. Biol. Univ. Mich., 26:1-19.

BLANDAU, R. J., and E. S. JORDAN

1941 The effect of delayed fertilization on the development of the rat ovum. Amer. Journ. Anat., 68:275-91.

BRAMBELL, F. W. R.

1944 Reproduction of wild rabbit, Oryctolagus cuniculus. Proc. Zool. Soc. London, 114:1-45.

BRAMBELL, F. W. R., and I. H. MILLS

1948 Studies on sterility and prenatal mortality in wild rabbits. IV. The loss of embryos after implantation. Journ. Exper. Biol., 25:241-69.

BRANDT, A. E.

1933 The analysis of variance in a "2 x s" table with disproportionate frequencies. Journ. Amer. Stat. Assoc., 28:164-73.

CLARK, FRANK H.

1936 The estrous cycle of the deer-mouse, Peromyscus maniculatus. Contrib. Lab. Vert. Genetics Univ. Mich., 1:1-7.

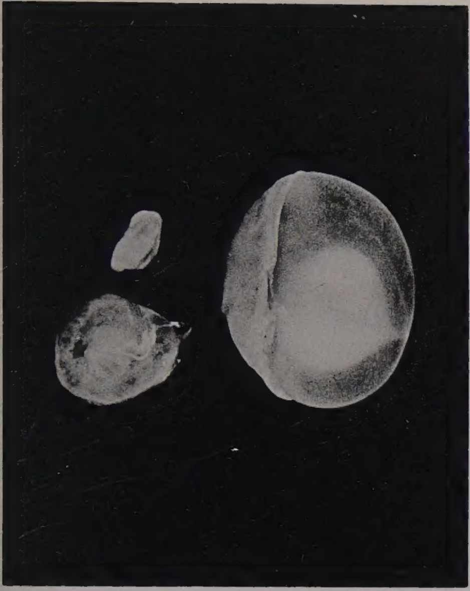
CLARK, PHILIP J., and T. T. LIU

MS Postnatal mortality in some laboratory-bred Peromyscus.

CORNER, G. W.

1923 The problem of embryonic pathology in mammals with observations upon intra-uterine mortality in the pig. Amer. Journ. Anat., 31:523-45.

PLATE I



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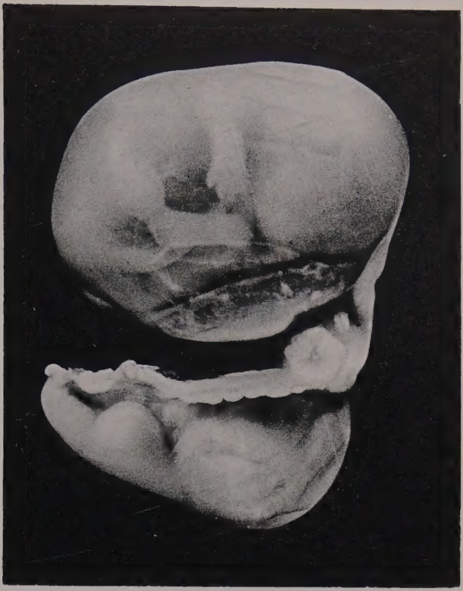

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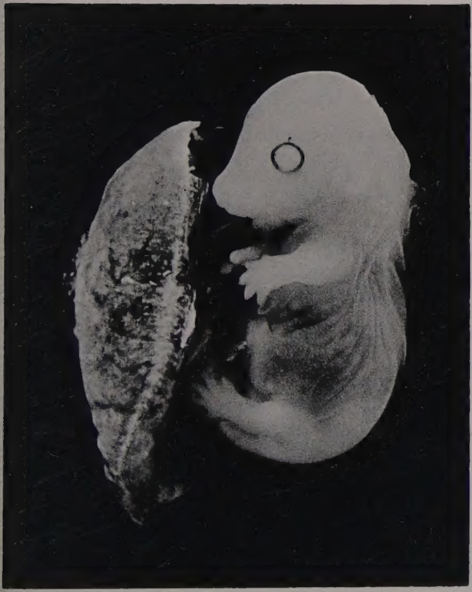

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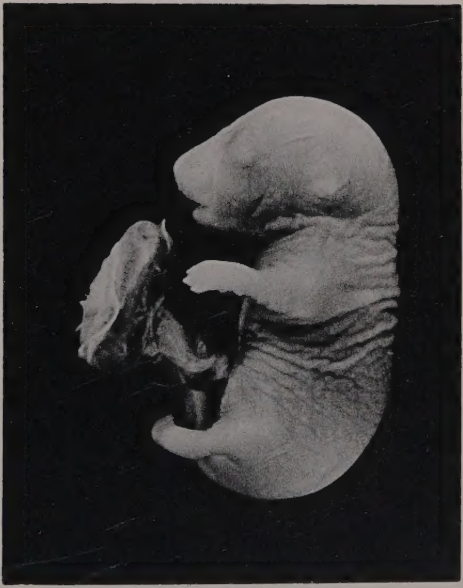

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FIG. 1. Three resorbed embryos. Upper left, material in which no placental or embryonic tissues can be distinguished; lower left, partly resorbed placenta with remnant of embryonic membranes; right, partly resorbed placenta with complete embryonic membranes containing rudimentary embryo (all enlarged). FIG. 2. Preserved uterus: a normal fetus in one horn and two resorbed embryos in the other (enlarged). FIG. 3. Dead fetus from the cross P. polionotus leucocephalus × P. maniculatus blandus, showing the disproportionally large placenta (enlarged). FIG. 4. Fully developed fetus with normal placenta from the cross P. maniculatus blandus inter se (enlarged).

DICE, LEE R.

1933 Fertility relationships between some of the species and subspecies of mice in the genus Peromyscus. Journ. Mammalogy, 14:298-305.

1947 Deer mice. Methods for rearing of Peromyscus. In: The UFAW handbook on the care and management of laboratory animals. London: Bailliere, Tindall and Cox. Pp. 171-77.

DOBZHANSKY, THEODOSIUS

1941 Genetics and the origin of species. 2d ed.; New York: Columbia Univ. Press.

FALCONER, D. S.

1952 A totally sex-linked gene in the house mouse. Nature, 169:664.

FISHER, R. A.

1948 Statistical methods for research workers. 10th ed.; New York: Hafner Publishing Company.

HALL, OCTAVIA, and DAVID E. DAVIS

1950 Corpora lutea counts and their relation to the number of embryos in the wild Norway rat. Texas Rpts. on Biol. and Med., 8:564-82.

HAUSCHKA, THEODORE S., MARGARET B. GOODWIN, and ELIZABETH BROWN

1951 Evidence for a sex-linked lethal in the house mouse. Genetics, 36:235-53.

HOLLANDER, W. F., and L. C. STRONG

1950 Intra-uterine mortality and placental fusion in the mouse. Journ. Exper. Zool., 115:131-50.

LIU, T. T.

1953 The measurement of fertility and its use as an index of reproductive isolation among certain laboratory stocks of Peromyscus. Contrib. Lab. Vert. Biol. Univ. Mich., 59:1-12.

MAC DOWELL, EDWIN C.

1924 A method of determining the prenatal mortality in a given pregnancy of a mouse without affecting its subsequent reproduction. Anat. Rec., 27:329-36.

OSGOOD, W. H.

1909 Revision of the mice of the American genus Peromyscus. U. S. Dept. Agric., N. Amer. Fauna, 28:1-285.

SPUHLER, J. N.

1948 On the number of genes in man. Science, 108:279-80.

WATSON, M. L.

1942 Hybridization experiments between Peromyscus polionotus and Peromyscus maniculatus. Journ. Mammalogy, 23:315-16.

WHITE, M. J. D.

1945 Animal cytology and evolution London: Cambridge Univ. Press.

YERUSHALMY, G.

1939 Age of father and survival of offspring. Human Biol., 11:342-56.

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